Flow Cytometer Based Biosensor for In-Field Cell Analysis

Cynthia A. Heil and Gabriel A. Vargo
College of Marine Science
University of South Florida
St. Petersburg, FL 33701-5016
phone (727) 553-3612 fax (727) 553-1189 email cheil@seas.marine.usf.edu
phone (727) 553-1167 fax (727) 553-1189 email vargo@seas.marine.usf.edu

David P. Fries and Ziaoling Ding
Center for Ocean Technology
College of Marine Science
University of South Florida
St. Petersburg, FL 33701-5016
phone (727) 553-3990 fax (727) 553-3967 email dfries@seas.marine.usf.edu
phone (727) 553-3990 fax (727) 553-3667 email xding@seas.marine.usf.edu

David F. Millie
Florida Marine Research Institute
Florida Fish & Wildlife Conservation Commission
Florida Institute of Oceanography
St. Petersburg, FL 33701
Phone (727) 896-8626 ext. 1527 fax (727) 550-4220 email David.Millie@fwc.state.fl.us

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LONG-TERM GOALS

The overall long-term goal of this research is the development of a self contained, fieldable microfluidic flow cytometer unit for in field cell screening and biological microorgansim population assessment. Development of the flow cytometer unit can provide the instrument platform with which specific target organisms of interest within a mixed population can be detected when a suitable labeling technology is becomes available. Targeted organisms can be those of interest to homeland security (e.g. toxic phytoplankton species, marine pathogens, etc) or those species of interest within models of nearshore coastal processes. Miniaturization of flow cytometry technology also allows adaptation of this technology for inclusion in AUVs with subsequent deployment and detection of the targeted organisms under *in situ* coastal conditions. The fast response nature of this flow cytometer cell screener will provide greater detection and response capabilities as well as, upon AUV deployment, possible support to toxicological assessments in an automated and highly controlled manner, all at a much lower cost than existing available technology.

OBJECTIVES

The main focus of this effort is to advance the design, fabrication, scientific applications and field-testing of a microfluidic device that will quantify and sort samples of particles (e.g. phytoplankton or bacterial cells) with specific characteristics from a mixed sample and to adapt this technology for

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14. ABSTRACT

The overall long-term goal of this research is the development of a self contained, fieldable microfluidic flow cytometer unit for in field cell screening and biological microorgansim population assessment. Development of the flow cytometer unit can provide the instrument platform with which specific target organisms of interest within a mixed population can be detected when a suitable labeling technology is becomes available. Targeted organisms can be those of interest to homeland security (e.g. toxic phytoplankton species, marine pathogens, etc) or those species of interest within models of nearshore coastal processes. Miniaturization of flow cytometry technology also allows adaptation of this technology for inclusion in AUVs with subsequent deployment and detection of the targeted organisms under in situ coastal conditions. The fast response nature of this flow cytometer cell screener will provide greater detection and response capabilities as well as, upon AUV deployment, possible support to toxicological assessments in an automated and highly controlled manner, all at a much lower cost than existing available technology.

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operation in AUVs under *in situ* conditions. Specific objectives include: 1) development of flow cytometry lab chips to serve as a platform for multiple and/or serial assays which can potentially be adapted for detection of any organism of interest. 2) adaptation of the unit and chip technology for examination of *in situ* aquatic populations using three representative, potentially significant marine organisms such as the toxic red tide dinoflagellate *Karenia brevis* and the fecal coliform enterovirulent pathogen *Escherichia coli*, found in sewage contaminated coastal waters, 3) integration of instrument with available *in situ* sampling platforms (e.g. Bottom-Stationed Ocean Profilers [BSOPs], *Nektor/Ranger* AUVs, and the real-time, instrument–based, coastal monitoring program '*Physical Oceanographic Real-Time System* [PORTS]) and 4) calibration and field testing of the resting system in the coastal waters of western Florida. During year one of the project, objectives one and two are the primary focus of the project.

APPROACH

Current flow cytometry is restricted to 'benchtop' or full-sized laboratory models which are generally dedicated to single use technology. Development of readily adaptable chip technology will allow the flow cytometer unit to be adaptable to a wide variety of potential uses limited only in development of cell recognition technology and include detection of toxic and harmful organisms *in situ* (e.g. cholera) and the generation of data for improvement of coastal process models. The proposed unit will be portable and of a sufficiently compact size to be adaptable to a variety of AUV platforms.

We have previously used an Agilent Bioanalyzer for chip dependent biomolecular assays (e.g. molecular characterization for DNA/RNA and proteins) and the base optics, electronics, glass microfluidic chips and the control software are all included in the system package. An extension of the microchannel technology is to use the technology for cell sorting and screening in a flow cytometric mode (Gawad, 2001; Wolff, 2001; De Gasperis, 1999) The chip provides cell fluorescence parameters within a chip format. The miniature analysis system developed within this project will be a simple, robust pressure-driven microchip for fluid control and probe staining for cell selection along with integrated optical probe detection. Figure 1 shows a base architecture for parallel channel cell analyzer.

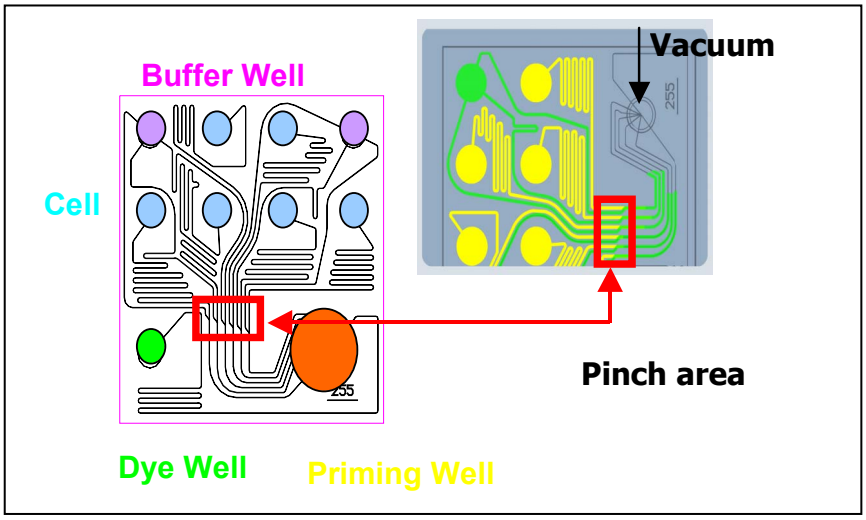

Fig. 1. Overall architecture of a multi-channel micro-chip Flow Cytometer showing cell, buffer, dye and primer wells interconnect with microfluidic channel logic. To the right is an enlarged region of the hydro-dynamic focusing region and vacuum access port

Two formats for the cell sorting biosensor are being pursued, initially a disposable analysis chip and then a continuous field monitor capable of repetitive sampling. The field system, upon development, will constitute a self-contained automated canister containing the flow cytometer, fluid lines, valves and electronic controls. A separate physical filtering system for sample prep will be co-located with the cell analyzer. The system will use a separate battery pack for all power requirements and have a functional bulkhead capable of accepting standard electrical signals (RS-232, Ethernet etc…) and standard fluidic interconnects (1/16" tubing). The instrument potentially can be deployed as a standalone in an independent water deployment or will be coupled to a small format AUV such as Nekton Research's Ranger or the Remus vehicle.

WORK COMPLETED

This project is currently in it's initial stages of research. A test organism, the toxic red tide dinoflagellate *Karenia brevis* has been received from the culture collection of the Florida Fish and Wildlife Conservation Commission and established in clonal culture at USF College of Marine Science. Currently cultures volumes are being ramped up to produce the large cell biomass required for chip experimentation and development. Two sources for whole cell, species specific fluorescence probes for this species (which do not cross-react with other *Karenia* spp.) have been identified: Dr. Allison Haywood, Cawthorne Institute, NZ and Dr. Peter McGuire, University of Florida College of Medicine and discussions for probe access are underway. The feasibility of using *E. coli* as a test organism discussed with Dr. John Paul, USF.

An Agilent Flow Cytometer unit is currently at USF Center of Technology. Currently, adaptation of the ship technology (the well and fluid channel array) to accommodate the larger oblate spheroid cell shape of the target organism is underway. We have made initial success with the creation of microchannels in liquid crystal polymer (Figure 2-right) and in also bonding glass to liquid crystal polymer. The bonding is needed in order to create a window for fluorescence detection. Bond testing has been completed and the bond between the thermoplastic liquid crystal polymer and the glass was found to survive water immersion for 1 week. The test was terminated at 1 week. Further testing of long term adhesion in salt water will be enacted. The flow channel system was also further integrated with surface mount fluidic connectors to allow planar chip to capillary tubing integration. The flow channel system is shown in Figure 2. We now have an adaptive chip creating toolbox for making the proper architecture for any target cell plus the material set to make low cost, integrated microfluidic systems. Specific architectures for *Karenia brevis* are is the next activity.

We have also completed an initial modeling of the light scattering expected from *Karenia brevis* and are exploring the potential of adding scattering measurements in addition to tagging as a mode of detection. The scattering can be used as a trigger for subsequent probe tagging and species specific detection. This will enable a higher speed probing of the water but still retain the probe specific advantage of the analysis.

RESULTS

None to date due to the start date of the project.

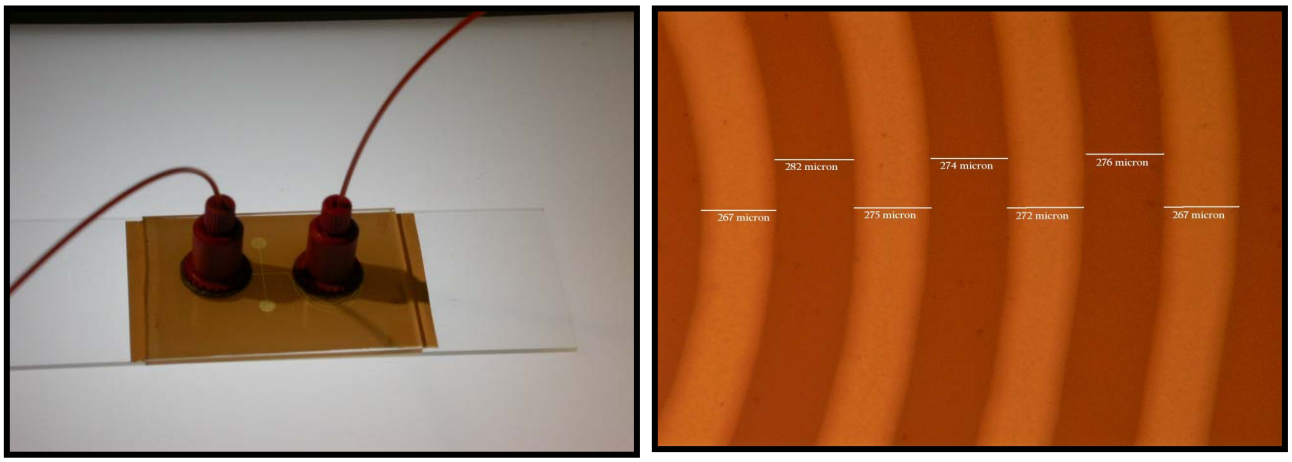

Figure 2. Flow channel chip made with Liquid Crystal Polymer up layer and glass lower layer. The polymer has channels micromachined into the polymer (shown right) and the system has surface mounted fluidic interconnects to allow fluid introduction from tubing connected to a pumping system. The glass underside permits the interrogation of the flow channels for fluorescence of the molecular probes attached to the flow focused cells.

IMPACT/APPLICATIONS

The resultant cell sorting technology will provide an added biocellular component to both AOSN and bio-physical models that support AOSN. The fast response nature of the cell screener will provide greater detection and response capabilities to sensing nodes and adaptive sampling AUV's under within AOSN. Once deployed the technology will also provide possible support to toxicological assessments in an automated and highly controlled manner, all at a much lower cost than existing available technology.

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